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(71) Applicant *(for all designated States except US)*: **PL BIO CO., LTD.** [KR/KR]; 570-1, Shinlim-dong, Kwanak-ku, Seoul 150-010 (KR).

(71) Applicant and
(72) Inventor *(for US only)*: **PAEK, Kyung-Soo** [KR/KR]; Pacific House B-203 129-2, Anam 5-ka,, Sungbuk-ku, 126-075 Seoul (KR).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **LEE, Yeonhee** [KR/KR]; Pacific House B-203, 129-2, Anam 5-ka, Sungbuk-ku, Seoul 126-075 (KR).

(74) Agent: **YOU ME PATENT AND LAW FIRM**; Teheran Building, 825-33 Yoksam-dong, Kangnam-Ku, Seoul 135-080 (KR).

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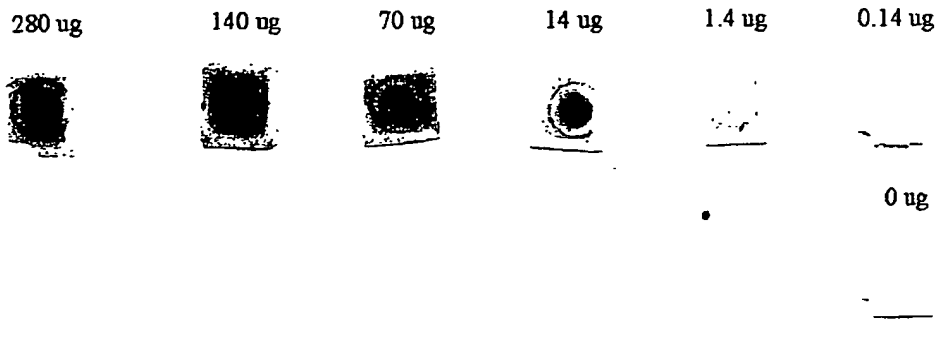

(57) Abstract: The present invention relates to lactic acid bacteria inhibiting the activity of *(Helicobacter pylori)*. This invention provides lactic acid bacteria, which suppresses *(Helicobacter pylori)* adherence on the gastric mucous membrane and *(Helicobacter pylori)* growth, and the lactic acid bacteria selected from the group consisting of *(Lactobacillus coprophilus)* PL 9001(KCCM-10245), *(Enterococcus durans)* PL 9002(KCCM-10246), *(Streptococcus faecalis)* PL 9003(KCCM-10247) *(Lactobacillus coprophilus)* PL 9004(KCCM-10248). *(Lactobacillus fermentum)* PL 9005 (KCCM-10250), and *(Lactobacillus fermentum)* PL 9006 (KCCM-10251). The lactic acid bacteria of the invention is applied to gastric ulcer restrainers, food additives, drugs for the prevention or treatment of *(Helicobacter pylori)*, drugs for bacillus causing food poison, or drugs for the prevention or treatment of infectious bacteria.

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LACTIC ACID BACTERIA WITH INHIBITING ACTIVITIES ON *HELICOBACTER PYLORI*

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to lactic acid bacteria with inhibiting activities on *Helicobacter pylori*, more particularly, to lactic acid bacteria with inhibitory activity for growth and adherence of *Helicobacter pylori* to gastric mucous membrane causing ulcer of stomach and growth.

(b) Description of the Related Art

Helicobacter pylori is a gram-negative microorganism. In 1983, Warren and Maeshall first isolated and characterized *Helicobacter* in the human gastric mucous membrane. *Helicobacter* initially was called *Campylobacter pyloridis,* and was later called *Helicobacter pylori* after morphology in the body and name of the habitat, pylorus.

Helicobacter pylori cause gastritis and gastric carcinoma (Dubois, A. 1995. Spiral bacteria in the human stomach: the gastric Helicobacters. *Emerg. Infect. Dis.* 1: 79-85; Slomiany, B. L. and A. Slomiany. 1992. Mechanism of *Helicobacter pylori* pathogenesis: focus on mucus. *J. Clin. Gastroenterol.* 14 Suppl 1 S114-21). Once *Helicobacter pylori* infects, it remains for several decades and is not eliminated naturally and thus, *Helicobacter pylori* is a major cause of chronic gastritis.

Helicobacter pylori infect through the intake of food and *Helicobacter pylori* attach to the gastric mucous membrane and the duodenal mucous membrane. A disease-causing factor of *Helicobacter pylori* is a urease secreted for surviving in highly acidic condition of stomach, a flagellum for maintaining mobility and the outer membrane protein having adherence to the gastric mucous membrane.

In the attachment of *Helicobacter pylori* to the human gastric epithelium, *Helicobacter pylori* binds the same antigens identified by antigens of red blood cells (Alkout, A. M., C. C. Blackwell, D. M. Weir, I. R. Poxton, R. A. Elton, W. Luman, and K. Palmer. 1997. *Gastroenterol.* 112: 1179-1187; Boren, T., P. Flak, K. A. Roth, G. Larson, and S. Normark. 1993. *Science* 262: 1892-1895; Clyne, M. and B. Drumm. 1997. *Gastroenterol.* 113: 72-80). Antigens like the Lewis antigen isolated in human blood type O, are identified in the gastric mucous membrane. Therefore, gastritis breaks out mostly in blood type O cases (Heneghan, M. A., A. P. Moran, K. M. Feeley, E. L. Egan, J. Goulding J, C. E. Connolly, and C. F. McCarthy. 1998. *FEMS Immunol. Med. Microbiol.* 20: 257-266; Kobayashi, K., J. Sakamoto, Y. Yamamura, T. Kito, H. Inagaki, T. Watanabe, and H. Nakazato. 1991. *Nippon Geka Gakkai Zasshi* 92: 813-819; Kobayashi, K., J. Sakamoto, Y. Kito, Y. Yamamura, T. Koshikawa, M. Fugita, T. Watanabe, and H. Nakazato. 1993. *Am. J. Gastroenterol.* 88: 919-924).

For the removal of *Helicobacter pylori*, antibiotic drugs, restrainers on proton pumping, and gastric acid removers have been used. It is difficult

that *Helicobacter* was cultured with a large scale, thereby development of a vaccine using the entire germ remains unsuccessful. The method using antibiotic drugs has a side effect in that the *Helicobacter pylori* become resistant to the antibiotic and the possibility of re-infection is not prevented. The method using stomach acid remover that suppresses stomach acid secretion is a not basic solution. In addition, although the vaccine using urease has been developed, it is not effective. In the future, the development of a vaccine against *Helicobacter pylori* will be difficult, due to its complex culture conditions, which make it difficult to determine an active area for a vaccine.

Lactobacillus produce lactate and an unknown material, and it inhibit *Helicobacter pylori* growth. In the case of *Lactobacillus salivarius*, the culture solution is able to inhibit growth of *Helicobacter pylori* (Aiba, Y., N. Suzuki, A. M. Kabir, A. Takagi, and Y. Koga. 1998 *Am. J. Gastroenterol.* 93: 2097-2101). Also, *Lactobacillus* inhibits the binding between the Lewis antigen and *Helicobacter pylori* (Lee, Y., E. Shin, J. Lee, and J.H. Park. 1999. *Lactobacillus acidophilus* inhibits the *Helicobacter pylori* adherence. *J. Microb. Biotech.* 9: 794-797).

SUMMARY OF THE INVENTION

Accordingly, the present invention is designed by the necessities of the prior art, and it is an object of the present invention to provide bacteria that suppress stomach ulcers.

Also, it is an object to provide bacteria that inhibits the adherence of *Helicobacter pylori*. .

Also, it is an object to provide bacteria that inhibits the activity of *Helicobacter pylori*.

Also, it is an object to provide bacteria that inhibits the growth of *Helicobacter pylori*.

Also, it is an object to provide bacteria that inhibits the growth of bacillus causing food poisoning.

Also, it is an object to provide bacteria that inhibits the growth of anaerobic bacteria or bacillus causing acne.

In order to achieve these objects, the present invention also provides a lactic acid bacteria containing inhibitory activity on *Helicobacter pylori* adherence of the gastric mucous membrane. More preferably, the lactic acid bacteria is at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus* coprophilus PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

The present invention also provides a medicine for the prevention or treatment of infectious bacillus by the use of lactic acid bacteria. The bacillus is *Helicobacter pylori*, bacillus causing food poisoning, anaerobic bacteria or bacillus causing acne.

The present invention also provides a cosmetic composition containing lactic acid bacteria or spent culture of lactic acid bacteria.

The present invention also provides a food additive containing lactic acid or spent culture of lactic acid bacteria.

The present invention also provides a food prepared by fermentation with lactic acid bacteria.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a slide showing *Helicobacter pylori* attachment to glycolipid isolated from red blood cells, wherein the greater the level of glycolipid (0 ug, 0.14 ug, 1.4 ug, 14 ug, 70 ug, 140 ug, 280 ug), the greater the degree of attachment.

FIG. 2 is TLC plate showing that lactobacillus inhibit *Helicobacter pylori* adherence on glycolipid.

FIG. 3 shows that materials produced from PL bacteria, suppress the growth of *Helicobacter pylori*.

FIG. 4 is a schematic diagram showing the diameter of the growth-inhibited-zone for *Helicobacter pylori*.

FIG. 5 is a graph showing the growth-inhibited degree of *Helicobacter pylori* by spent culture of PL bacteria.

FIG. 6 is a graph showing growth inhibition of bacillus causing food

poisoning by *Lactobacillus coprophilus* PL 9001.

FIG. 7 is a graph showing growth inhibition of bacillus causing food poisoning by *Enterococcus durans* PL 9002.

FIG. 8 is a graph showing growth inhibition of bacillus causing food poisoning by *Streptococcus faecalis* PL 9003.

FIG. 9 is a graph showing growth inhibition of bacillus causing food poisoning by *Lactobacillus coprophilus* PL 9004.

FIG. 10 is a graph showing growth inhibition of bacillus causing food poisoning by *Lactobacillus fermentum* PL 9005.

FIG. 11 is a graph showing growth inhibition of bacillus causing food poisoning by *Lactobacillus fermentum* PL 9006.

FIG. 12 is a graph showing growth inhibition of bacillus causing acne by PL bacteria.

FIG. 13 is a graph showing effect of immune improvement by PL bacteria.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention will now be explained in more detail.

The present invention provides bacteria, inhibiting the adherence of *Helicobacter pylori* to the gastric mucous membrane. The bacteria are lactic acid bacteria and preferably *Lactobacillus sp.* or *Enterococcus sp.* More preferably, the bacteria are *Lactobacillus coprophilus*, *Enterococcus durans*, *Streptococcus faecalis* and *Lactobacillus fermentum,* and most preferably, are *Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL

9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005 and *Lactobacillus fermentum* PL 9006. The *Streptococcus faecalis* is identical to *Enterococcus faecalis* and then it was termed as *Streptococcus faecalis* PL 9003.

The PL bacteria (*Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005 and *Lactobacillus fermentum* PL 9006) are internationally deposited in the Korean culture center of microorganisms and given an accession number. The accession number given by the international depositary authority are KCCM-10245 for *Lactobacillus coprophilus* PL 9001, KCCM-10246 for *Enterococcus durans* PL 9002, KCCM-10247 for *Streptococcus faecalis* PL 9003, KCCM-10248 for *Lactobacillus coprophilus* PL 9004, KCCM-10250 for *Lactobacillus fermentum* PL 9005 and KCCM-10251 for, *Lactobacillus fermentum* PL 6.

Also, the present invention provides a gastric ulcer restrainer. The gastric ulcer restrainer contains *Lactobacillus sp.* or *Enterococcus sp.* and more preferably, contains the PL bacteria. The *Lactobacillus sp.* or *Enterococcus sp.* have inhibitory activity on *Helicobacter pylori* adherence to the gastric mucous membrane as well as for *Helicobacter pylori* growth. These effects are confirmed with *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus*

fermentum PL 9006 (KCCM-10251).

Also, the PL bacteria produce materials that inhibit the growth of *Helicobacter pylori* and adherence to the gastric mucous membrane, and thus, spent culture of the PL bacteria can be used for the gastric ulcer restrainer. The PL bacteria have antibiotic-resistant activity, acid-resistant activity, and bile-resistant activity, and are stable *in vivo*, whereby the PL bacteria can be provided as raw bacteria, dehydrated bacteria and dead bacteria.

The gastric ulcer restrainer of the present invention can be adjusted for use. The drug is administered orally, by injection, and ointment. Preferably, the drug would be administered orally or by injection; but most preferably, the drug would be administered orally. The gastric ulcer restrainer can be prepared as a single compound drug or complex drug and the complex drug can contain more pharmaceutically acceptable material. It is also reasonable that the blending ratio of the PL bacteria is controlled by using methods applicable to the type of drug.

The spent culture of PL bacteria is preferably solution without bacteria that is prepared by filtering or centrifuging culture fluid, and more preferably, the spent culture is a supernatant solution prepared by inoculating the PL bacteria in MRS liquid broth, culturing at 37 ℃, for 16 hr-48hr, and centrifuging.

Also, *Lactobacillus sp.* and *Enterococcus sp.* can be applied to inhibit the activity of other bacilli. More preferably, the PL bacteria (PL9001,

PL9002, PL9003, PL9004, PL9005, and PL9006), fragmented cell wall of the PL bacteria or spent culture of the PL bacteria can be used to inhibit the growth of bacillus causing food poisoning, anaerobic bacteria, bacillus causing acne and so on.

The anaerobic bacteria are general disease-causing germs. The anaerobic bacteria prefer tetanus bacteria, gas gangrene bacteria and *Clostridium sp.*, and more preferably, *Clostridium sp.*

The bacillus causing food poisoning is a listeria, a dysentery bacillus, diarrhea bacillus, 0157, and bacillus mediated food poisoning.

Also, *Lactobacillus* sp. and *Enterococcus* sp. have activity for improving immunity and can be applied to improve health.

Also, the *Lactobacillus* sp. and the *Enterococcus* sp. of the present invention is preferably used for feed, feed additives, food, food additives, medicine, or cosmetic composition. Moreover, the cell wall prepared by grinding the PL bacteria, raw, dead and dehydrated bacteria, and spent culture, are contained. The *Lactobacillus* sp. and the *Enterococcus* sp. of the present invention underwent a toxic test, which confirmed that the lactic acid bacteria were safe in vivo.

The food or food additives prefer yogurt, baby food, dairy goods, cheese, Kimchi, drinks, or food and is prepared by fermenting with the *Lactobacillus* sp. or the *Enterococcus* sp. of the present invention.

The medicine contained the *Lactobacillus* sp. or the *Enterococcus* sp. of the present invention is preferably formulated as a solution, powder, solid,

or capsule and most preferably, is a capsule type prepared by dehydrating.

The cosmetic composition can be used for massage, pack, solution type ointment, or as an additive in order to prevent and treat bacillus, such as acne. The cosmetic composition is preferably for external application.

Also, the present invention provides a material for inhibiting the growth of harmful bacteria. The material for inhibiting the growth of harmful bacteria is able to suppress the growth of *Helicobacter pylori*, anaerobic bacteria, bacillus causing acne or bacillus causing food poisoning and is prepared as spent culture in bacillus culture. Thus, the material for inhibiting the growth of harmful bacteria produced by *Lactobacillus* sp. or *Enterococcus* sp., can also be used for the treatment and prevention of bacteria.

As mentioned above, *Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004. *Lactobacillus fermentum* PL 9005, and *Lactobacillus fermentum* PL 9006 of the present invention suppress the growth and adhesion of *Helicobacter pylori*. Also, PL bacteria, fragment of PL bacteria, or spent culture of PL bacteria is able to be used for the treatment and prevention of bacteria. PL bacteria, and spent culture of PL bacteria have activity of inhibiting the growth of bacillus causing food poisoning, bacillus causing acne and anaerobic bacteria, and improving effect of immunity, thereby PL bacteria, and spent culture of PL bacteria can be used for treatment and prevention.

The present invention will be explained in more detail with reference to the following Examples. However, the following Examples are to illustrate the present invention and the present invention is not limited to them.

EXAMPLE

MRS+BPB are prepared with MRS broth (Difco, bacto proteose peptone No.3 10 g, bacto beef extract 10 g, bacto yeast extract 5 g, bacto dextrose 20 g, polysorbate 80 g, ammonium citrate 2 g, sodium acetate 5 g, magnesium sulfate 0.1 g, manganese sulfate 0.05 g, dipotassium phosphate 2 g /L) containing 0.002 % of bromophenol blue. Kimchi samples are diluted with 10 X pepton solutions. 0.1 mL of the diluted solution are inoculated in MRS+BPB medium and spread. The feces from an infant were picked by a cotton swab and inoculated in MRS+BPB medium. After incubation for 3-4 days in an incubator (25 ℃), *Lactobacillus sp.* were observed and isolated according to the formed colonies.

In order to distinguish a group of *Lactobacillus sp.*, MRS solid media was added with BPB which displays yellow color in pH 3.0 and purple color in pH 4.6, and *Lactobacillus sp.* was classified according to a coloration by BPB. The coloration was determined by producing degrees of lactic acid, pH-resistant, and the length of life. *P. acidolactic* and *S. faecalis* are normal lactic acid bacterium, *L. mesenteroides* and *L.* brevis are abnormal lactic acid bacterium, and *L. plantarum* are random lactic acid bacterium. Due to ferment lactic acid, the production of a lot of lactic acid, *S. faecalis* has

formed a white colony where the medium of it has changed to light yellow. *L. mesenteroides* ferment abnormally lactic acid and produce a low level of lactic acid, the colony is deep blue as a whole and has a small size without a ring. *Lactobacillus sp.* have a light blue ring, being deep blue in center, or generally white color, and the size of the colony is large. *P. acidolactic* and *L. mesenteroides* express a deep-blue for a short life time and low pH. For example, *L. mesenteroides* cannot growth below pH 4.8.

All bacteria of the present invention formed a white colony of over 0.3 mm (diameter) and are classified into *Lactobacillus sp.*

Bacteria were isolated from single colony and, according to Bergy's manual of systematic bacteriology, exhibited morphological and biochemical properties. After performed by gram stain and catalase-reaction, the bacteria were observed in an API system (La Balme-les-Grottes, France). After the colony was picked with the cotton wrap, it was floated with 2 ml of distilled water. The floating solution was added to the API 50 CHL media and mixed. Each 200 ul of the mixture was inoculated into 50 tubes, was covered with mineral oil, and then was incubated at 37℃, for 48 h. The fermentation pattern of the 49 kinds of saccharide were analyzed with API 50 CH and API 50 CHL, the resulting data was fed into the ATB identification computer system (bio Merieux France), and then bacterium was identified. The result of the first bacterium among the isolated microorganisms is presented in Table 1.

[Table 1]

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	- Galactose	- D-Mannoside	- Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	+ Glucosamine	- Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	+ Amygdaline	- Inuline	- D-Fucose
- L-Arabinose	- L-Sorbose	+ Arbutine	- Melezitose	- L-Fucose
- Ribose	- Rhamnose	+ Esculine	- D-Raffinose	- D-Arabitol
+ D-Xylose	- Dulcitol	+ Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	+ Cellobiose	- Glycogene	+ Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	- Lactose	+ Gentiobiose	- 5-Gluconate

The first bacterium was identified 99.9 % to *Lactobacillus coprophilus* and 0.1 % to *Lacto. brevis*. 16S rRNA of the first bacterium was sequenced with 16S rRNA gene kit (Perkin Elmer Applied Biosystem) and sequence was shown in Sequence No. 1. The base sequence of Sequence No. 1 is identical to 16S rRNA base sequence of *Lactobacillus coprophilus* as the result of BLAST search (http://www.ncbi.nlm.nih.gov/blast) other name of the *Lactobacillus coprophilus* is *Weissella confusa* and *Lactobacillus confuses*. The bacterium was deposited under Lactobacillus 9001(KFCC-11240) in the Korean federation of culture collections, and internationally deposited under *Lactobacillus coprophilus* PL 9001(KCCM-10245) in the Korean culture center of microorganisms.

The second bacterium was characterized with API STREP Kit and the result was presented in Table 2.

[Table 2]

VP	+	ARA	+
HIP	-	MAN	+
ESC	+	SOR	-
PYRA	+	LAC	-
AGAL	-	TRE	-
GUR	-	INU	-
GAL	-	RAF	-

PAL	-	AMD	-
LAP	-	GLYG	-
ADH	+	- HEM	-
RIB	+		

For the second bacterium was not classified, 16S rRNA base sequence was analyzed. 16S rRNA base sequence of the second bacterium is Sequence No. 2, and identical to that of *Enterococcus durans*. According to the sequencing result, the second bacterium was called *Enterococcus durans* PL 9002, was deposited under *Enterococcus durans* PL9002 (KFCC-11241) in the Korean federation of culture collections, and internationally deposited under *Enterococcus durans* PL 9002(KCCM-10246) in the Korean culture center of microorganisms.

The result of the third bacterium is presented in Table 3.

[Table 3]

VP	+	ARA	-
HIP	-	MAN	+
ESC	+	SOR	+
PYRA	+	LAC	+
AGAL	-	TRE	+
GUR	-	INU	-
GAL	-	RAF	-
PAL	-	AMD	+
LAP	+	GLYG	-
ADH	+	- HEM	-
RIB	+		

The third bacterium was identified 99.1 % to *Streptococcus faecalis* (*Enterococcus faecalis*). The bacterium was deposited under *Streptococcus faecalis* 9003(KFCC-11242) in the Korea federation of culture collections, and internationally deposited under *Streptococcus faecalis* PL 9003(KCCM-10247) in the Korea culture center of microorganisms.

16S rRNA base sequence of the third bacterium was analyzed as Sequence No. 3, and identical to that of *Streptococcus faecalis* (*Enterococcus faecalis*).

The result of the fourth bacterium is presented in Table 4.

[Table 4]

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	- Galactose	- D-Mannoside	- Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	+ Glucosamine	- Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	+ Amygdaline	- Inuline	- D-Fucose
+ L-Arabinose	- L-Sorbose	- Arbutine	- Melezitose	- L-Fucose
- Ribose	- Rhamnose	+ Esculine	- D-Raffinose	- D-Arabitol
+ D-Xylose	- Dulcitol	+ Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	+ Cellobiose	- Glycogene	+ Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	- Lactose	+ Gentiobiose	- 5-Gluconate

The fourth bacterium was identified 98.2 % to *Lactobacillus. coprophilus* and 1.3 % to *Lacto. brevis*. Also, 16S rRNA base sequence of the bacterium was analyzed as Sequence No. 4, and identical to that of *Lactobacillus coprophilus* as the result of BLAST search (http://www.ncbi.nlm.nih.gov/blast). The bacterium was deposited under *Lactobacillus coprophilus* PL 9004(KFCC-11243) in the Korea federation of culture collections, and internationally deposited under *Lactobacillus coprophilus* PL 9-4(KCCM-10248) in the Korean culture center of microorganisms

The result of the fifth bacterium is presented in Table 5.

[Table 5]

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	+ Galactose	- D-Mannoside	+ Melibiose	- D-Turanose

- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	+ Glucosamine	- Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	- Amygdaline	- Inuline	- D-Fucose
- L-Arabinose	- L-Sorbose	- Arbutine	- Melezitose	- L-Fucose
+ Ribose	- Rhamnose	- Esculine	+ D-Raffinose	- D-Arabitol
- D-Xylose	- Dulcitol	- Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	- Cellobiose	- Glycogene	+ Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	- Lactose	- Gentiobiose	- 5-Gluconate

The fifth bacterium was identified 93.2 % to *Lactobacillus fermentum* and 6.7 % to *Leuoconostoc lactis*. Also, 16S rRNA base sequence of the bacterium was analyzed as Sequence No. 5, and identical to that of *Lactobacillus fermentum* as the result of BLAST search (http://www.ncbi.nlm.nih.gov/blast). The bacterium was deposited under *Lactobacillus fermentum* PL 9005(KCCM-10250) in the Korean culture center of microorganisms

The result of the sixth bacterium is presented in Table 6.

[Table 6]

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	+ Galactose	+ D-Mannoside	+ Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	- Glucosamine	+ Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	- Amygdaline	- Inuline	- D-Fucose
- L-Arabinose	- L-Sorbose	- Arbutine	- Melezitose	- L-Fucose
+ Ribose	- Rhamnose	- Esculine	+ D-Raffinose	- D-Arabitol
- D-Xylose	- Dulcitol	- Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	- Cellobiose	- Glycogene	- Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	+ Lactose	+ Gentiobiose	- 5-Gluconate

The sixth bacterium was identified 94.4 % to Lactobacillus fermentum and 5.4 % to *Lactobacillus lactis.* Also, 16S rRNA base sequence of the bacterium was analyzed as Sequence No. 6, and identical to that of *Lactobacillus fermentum* as the result of BLAST search

(http://www.ncbi.nlm.nih.gov/blast). The bacterium was deposited under *Lactobacillus fermentum* PL 9006(KCCM-10251) in the Korean culture center of microorganisms

TEST 1. Test for inhibiting adherence of *Helicobacter* pylori on the gastric mucous membrane.

(1) Preparation of bacteria

Helicobacter pylori (ATCC 43504) cultured for 48 hr on a Brucella solid medium [Brucella broth, fungizone (2.5 g/ml amphotericin B), and Skirrow's supplement (0.16 mg/ml polymyxin B, 5 mg/ml vancomycin, 2.5 mg/ml trimethoprim)] supplemented with 10% horse serum under 5-10% CO_2 incubator. Cultured cells were collected by scraping, washed twice with phosphate-buffered saline (PBS, pH 7.4), and then kept in 10 mM Tris-Cl buffer at -20℃ until used.

The bacteria prepared by EXAMPLE 1 referred as PL bacteria. The PL bacteria (*Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251)) were grown for 24 h, at 37℃ in MRS broth, collected by centrifugation, pellet washed 2-3 times with PBS(pH 7.4), and then kept in 10 mM Tris-cl at -20℃ until used.

(2) Extraction and purification of glycolipid

Glycolipid was isolated from human O-type red blood cells (RBCs),

as described in the report with a slight modification (Lee, Y., E. Shin, J. Lee, and J.H. Park. 1999. *J. Microb. Biotech.* 9: 794-797). The human O-type RBCs were dispersed in a minimum volume of water, repeated process of freezing at –70 ℃ and melting, and then destructed. After the lysate was fixed, the supernatant was removed. The remains collected and extracted as mentioned below. The remains were mixed with a chloroform-methanol mixture (2:1, v/v) and a lower phase (containing lipid) collected. The material of lipid layer was dried in a rotary vacuum evaporator. The dried solid was dissolved in chloroform containing 2 % of methanol and loaded on a column (bed volume = 20 ml) that was equilibrated with silicic acid (H_2SiO_3). The column fraction was collected by sequentially eluting with chloroform, acetone-methanol (3:1, v/v), and methanol. The methanol fraction was then dried in a rotary vacuum evaporator, dissolved in a minimum volume of methanol, and stored in microcentrifuge tube at –70 ℃.

(3) Competitive lipid binding assay

Competitive lipid binding assay was performed with a slight modification (Lee, Y., E. Shin, J. Lee, and J.H. Park. 1999. *J. Microb. Biotech.* 9: 794-797). The extracted glycolipid (14 ug/5 ul) was spotted on a thin layer chromatography plate. (TLC, Merck, Kreselgel60, EM Separations, Gibbstown, NJ, U.S.A) The plate was soaked in 10 mM Tris buffer (pH 7.6) containing 3 % gelatin for 2 h at 37℃ to prevent non-specific binding. The TLC plate was rinsed two times with the same buffer and was added culture solution of PL bacteria (OD_{600}=1, CFU 2.4 X 10^8). After 5 to 30 min, *H.*

pylori (2.0 X 10^8 CFU) was added and mixture was gently agitated for 2 hr at 37℃. In addition, except PL bacteria, the control plate was reacted with only *Helicobacter pylori* as described above, and agitated for 2 hr. The plate was rinsed 3 times with the same buffer for 10 min/time. The first antibody, rabbit antiserum raised against *H. pylori* was added to the buffer (1: 600) and further incubated for 2 hr at room temperature with gentle shaking. After the plate was washed to remove the first antibody (1: 1000), IgG, the second antibody conjugated with alkaline phosphatase, was added to the plate and the plate was incubated for 1 hr at room temperature. A chromogenic reaction was observed by adding BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate disodium salt/nitro blue tetrazolium chloride). Therefore, the chromogenic level shows the adherence degree of *Helicobacter pylori* on glycolipid derived from red blood cells.

FIG. 1 is TCL plate showing that bound *Helicobacter pylori* to glycolipid from red blood cells, the amounts of bound *H. pylori* increased linearly as the amount of spotted glycolipid (0 ug, 0.14 ug, 1.4 ug, 14 ug, 70 ug, 140 ug, 280 ug) increased.

FIG. 2 is TLC plate observed whether or not *Lactobacillus* inhibit adherence of *Helicobacter pylori* on glycolipid. PL bacteria (*Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251)) was

reacted with *Helicobacter pylori*. As shown in Fig. 2, PL bacteria of the EXAMPLE inhibited the adherence of *Helicobacter pylori*.

TEST 2. Test of inhibiting *Helicobacter pylori* growth.

(1) Test of inhibiting *Helicobacter pylori* growth using spent culture of PL bacteria.

The PL bacteria (*Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251)) cultured for 24 h, at 37℃ in MRS broth, and the supernatant was prepared spent culture by centrifugation.

Inhibition test of *Helicobacter pylori* growth using spent culture of PL bacteria was performed.

Wells were formed on Brucella solid media using a sterilized Pasteur pipet. *Helicobacter pylori* spread on the media and the well was added with the spent culture of PL bacteria. After incubating in a CO_2 incubator (5 % to 10 % CO_2) for 2 days, the diameter of the zone that *Helicobacter pylori* growth inhibited due to the inhibitory material of the spent culture, was measured.

FIG. 3 is a picture showing that the spent culture has growth inhibitory activity against *Helicobacter pylori* and FIG. 4 shows a diameter of the growth-inhibited-zone of *Helicobacter pylori* by the spent culture. Therefore, it was confirmed that PL bacteria prepared by the EXAMPLE,

secreted material that inhibited *Helicobacter pylori* growth.

(2) Test of inhibiting *Helicobacter pylori* growth by lyophilized spent culture of PL bacteria.

4 ml of MRS spent culture of PL bacteria was mixed with 2 ml of distilled MQ and the mixture was lyophilized. The lyophilized spent culture was suspended on 6 ml of skim milk (10 mg/ml) and after 1 ml of the solution was inoculated with 10 ul of *Helicobacter pylori* (OD_{625}=1.0), it was incubated for 1hr, at 37 ℃ CO_2 incubator. The incubated solution was centrifuged (5000 rpm, 10min) and supernatant was diluted half with distilled MQ. The quantity of urease of diluted solution was measured by indolphenol method. The quantity of urease is proportional to urease activity, and amount of *Helicobacter pylori* in the incubated solution was analyzed. Through these methods, growth inhibition of *Helicobacter pylori* by spent culture of PL bacteria was analyzed.

FIG. 5 is a graph showing the growth-inhibition degree of *Helicobacter pylori* by spent culture of PL bacteria, it was known that lyophilized spent culture of PL bacteria maintains the growth inhibition activity for *Helicobacter pylori*.

TEST 3. Toxicity test

The oral toxic test for the PL bacteria of EXAMPLE was based on the 'toxic test standard for drug (1999. 12. 22)' referred as Notification No. 1999-61 of the Korean Food and Drug Administration.

Tests were conducted on Sprague-Dawley rats, which were 5 weeks old (female : 100 ~ 120g / male : 110 ~ 130g). The rats were reared in 260 × 420 × 180 mm (W x L x H) cages at 23 ± 2 ℃ and 50 ± 10 an.

Referring to established rule No. 10 of the Korean Food and Drug Administration, when the value of LD_{50} is up to 5,000 mg/kg in an oral test, the material is low toxicity material in body. Therefore, in the experiment, to calculate the value of LD_{50} of PL bacteria, the dosage was set to 5,000 mg/kg (20 ml/kg B.W.) 5,000 mg/kg was the maximum dosage given to the rat to determine the toxicity of the material.

[Table 7]

Bacteria	Sex	number	Dosage(mg/kg B.W)	Dosage(ml/kg B.W)
I(PL 9001)	Male	5	5,000	20
	Female	5		
II(PL 9002)	Male	5	5,000	20
	Female	5		
III(PL 9003)	Male	5	5,000	20
	Female	5		
IV(PL 9004)	Male	5	2,500	20
	Female	5		
V(PL 9004)	Male	5	5,000	20
	Female	5		
VI(control)	Male	5	0	20
	Female	5		

After 14 days, no deaths were observed. Thus the value of LD_{50} could not be estimated.

Table 8 shows the weight change of the test group and there were no significant changes.

[Table 8]

Sex	Days	I	II	III	IV	V	VI
M	0	120.1± 3.10	120.2± 3.08	120.1± 3.08	120.4± 2.86	120.2± 3.25	120.1± 3.31

	3	142.6± 6.81	146.9± 4.15	140.5± 5.46	140.7± 8.85	145.4± 7.73	143.0± 3.68
	7	162.7± 7.96	161.0± 5.87	155.5± 2.92	156.6± 11.59	162.1± 5.64	159.8± 3.06
	14	212.3± 8.40	193.1± 18.03	194.9± 9.14	201.1± 22.72	195.5± 11.14	193.6± 16.24
F	0	114.0± 4.34	113.9± 4.11	113.9± 4.17	113.5± 4.41	113.5± 4.45	113.6± 4.50
	3	134.3± 6.81	132.6± 4.15	131.0± 5.46	132.5± 8.85	129.8± 7.73	129.2± 3.68
	7	155.4± 8.01	151.5± 5.69	149.5± 5.86	152.2± 7.55	148.6± 5.81	148.8± 6.57
	14	199.8± 8.16	192.7± 10.87	192.5± 10.0	193.8± 12.06	194.7± 7.55	192.6± 9.35

In addition, all the rats were examined to determine the cause of death, but there was no symptom visible to ordinary sight (Table 9).

[Table 9]

Organ		I	II	III	IV	V	VI
Brain	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Kidney-Left	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Kidney-Right	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Heart	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Lung	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Spleen	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Liver	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Stomach	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Intestine	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Pancreas	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Adrenal gland(left)	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Adrenal gland(right)	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Pituitary gland	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5

Ovary-L	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Ovary-R	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Other organs	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5

As shown above, PL bacteria of the EXAMPLE is safe without oral toxicity and side effects.

TEST 4. Growth inhibition test for bacillus causing food poisoning

MRS spent culture of each PL bacterium was mixed with 2 X concentrated BHI media. Each of *Salmonella typhimurium*, *Salmonella Enteriditis*, *E. coli O157:H7*, *Aeromonas hydrophila*, and *Listeria monocytogenes* cultured in BHI (Brain Heart Infusion: Brain Hear, Infusion form, 6.0 g, Peptic digest of animal tissue 6.0 g, sodium chloride 5.0 g, dextrose 3.0 g, pancreatic digest of gelatin 14.5 g, disodium phosphate 2.5 g) inoculated with 1 % final concentration on the mixture and incubated at 37℃. After 5 hr and 12 hr, the number of survived bacteria was measured. For measurement, *Listeria monocytogenes* (L.M) incubated in blood-agar plate. *Salmonella typhimurium* (S.T), *Salmonella Enteriditis* (S.E), *E. coli O157:H7* (O157) and *Aeromonas hydrophila* (A.H) incubated in MacConkey media and number of survived bacteria was measured at $O.D_{600}$.

Table 10 shows a $O.D_{600}$ of bacillus causing food poison by PL 9001 spent culture.

[Table 10]

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M + spent culture (PL9001)	0.059	0.069	0.068
S.T	0.053	0.538	0.748

S.T+ spent culture (PL9001)	0.063	0.073	0.072
S.E	0.053	0.572	0.966
S.E+ spent culture (PL9001)	0.059	0.073	0.07
O157	0.051	0.408	0.468
0157+ spent culture (PL9001)	0.06	0.074	0.065
A.H	0.077	0.418	0.432
A.H+ spent culture (PL9001)	0.069	0.093	0.083

FIG. 6 is a graph showing growth inhibition of bacillus causing food poisoning by *Lactobacillus coprophilus* PL 9001 and shows the result of table 10 (absorbance of $O.D_{600}$ at 0 hr calculate 100). As mentioned above in table 10 and Fig. 6, *Lactobacillus coprophilus* PL 9001 produced materials that inhibit the growth of bacillus causing food poisoning.

Also, *Enterococcus durans* PL 9002 inhibited the growth of bacillus causing food poisoning. Inhibition result shows in table 11 and Fig. 7.

[Table 11]

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M+ spent culture (PL 9002)	0.059	0.071	0.17
S.T	0.053	0.538	0.748
S.T+ spent culture (PL 9002)	0.064	0.073	0.196
S.E	0.053	0.572	0.966
S.E+ spent culture (PL 9002)	0.061	0.074	0.167
O157	0.051	0.408	0.468
0157+ spent culture (PL 9002)	0.59	0.073	0.17
A.H	0.077	0.418	0.432
A.H+ spent culture (PL 9002)	0.065	0.086	0.235

The inhibition effect of *Streptococcus faecalis* PL 9003 for growth of bacillus causing food poisoning was measured. The above table 12 shows $O.D_{600}$ of bacillus causing food poisoning in culture solution containing *Streptococcus faecalis* PL 9003, and it makes a graph (Fig 8) of data of table

12. *Streptococcus faecalis* PL 9003 produced materials that inhibited the growth of bacillus causing food.

[Table 12]

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M+ spent culture (PL 9003)	0.058	0.007	0.075
S.T	0.053	0.538	0.748
S.T+ spent culture (PL 9003)	0.063	0.074	0.084
S.E	0.053	0.572	0.966
S.E+ spent culture (PL 9003)	0.062	0.074	0.082
O157	0.051	0.408	0.468
0157+ spent culture (PL 9003)	0.058	0.07	0.071
A.H	0.077	0.418	0.432
A.H+ spent culture (PL 9003)	0.066	0.085	0.094

The inhibition effect of *Lactobacillus coprophilus* PL 9004 for the growth of bacillus causing food poisoning was measured. The above table 13 shows $O.D_{600}$ of bacillus causing food poisoning in culture solution containing *Lactobacillus coprophilus* PL 9004, and it makes a graph (Fig. 9) of data of table 13. *Lactobacillus coprophilus* PL 9004 produced materials that greatly inhibited the growth of bacillus causing food, but the inhibition effect was decreased.

[Table 13]

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M+ spent culture (PL 9004)	0.06	0.075	0.287
S.T	0.053	0.538	0.748
S.T+ spent culture (PL 9004)	0.063	0.071	0.266
S.E	0.053	0.572	0.966
S.E+ spent culture (PL 9004)	0.061	0.073	0.227
O157	0.051	0.408	0.468
0157+ spent culture (PL 9004)	0.062	0.073	0.267
A.H	0.077	0.418	0.432

A.H+ spent culture (PL 9004)	0.065	0.087	0.251

The inhibition effect of *Lactobacillus fermentum* PL 9005 for the growth of bacillus causing food poisoning was measured. The above table 14 shows $O.D_{600}$ of bacillus causing food poisoning in culture solution containing *Lactobacillus fermentum* PL 9005, and Fig 10 is a graph showing the growth inhibition for Listeria. *Lactobacillus fermentum* PL 9005 produced materials that greatly inhibited the growth of bacillus causing food.

[Table 14]

Condition	0 hr	5 hr	24 hr
L.M	0.055	0.487	0.335
L.M+ spent culture (PL 9005)	0.06	0.068	0.413
S.T	0.053	0.538	0.748
S.T+ spent culture (PL 9005)	0.065	0.074	0.434
S.E	0.053	0.572	0.966
S.E+ spent culture (PL 9005)	0.062	0.075	0.448
O157	0.051	0.408	0.468
O157+ spent culture (PL 9005)	0.06	0.077	0.389
A.H	0.077	0.418	0.432
A.H+ spent culture (PL 9005)	0.067	0.087	0.245

The inhibition effect of *Lactobacillus fermentum* PL 9006 for the growth of bacillus causing food poisoning was measured. The above table 15 shows a $O.D_{600}$ of bacillus causing food poisoning in culture solution containing *Lactobacillus fermentum* PL 9006, and Fig 11 is a graph showing the growth inhibition for Listeria. *Lactobacillus fermentum* PL 9006 produced materials that greatly inhibited the growth of bacillus causing food.

[Table 15]

Condition	0 hr	5 hr	24 hr
L.M	0.055	0.487	0.335
L.M+ spent culture (PL 9006)	0.058	0.068	0.283
S.T	0.053	0.538	0.748
S.T+ spent culture (PL 9006)	0.061	0.073	0.303
S.E	0.053	0.572	0.966

S.E+ spent culture (PL 9006)	0.065	0.069	0.428
O157	0.051	0.408	0.468
O157+ spent culture (PL 9006)	0.063	0.077	0.435
A.H	0.077	0.418	0.432
A.H+ spent culture (PL 9006)	0.067	0.087	0.112

As mentioned above, PL bacteria (*Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005 and *Lactobacillus fermentum* PL 9006) of the present invention produced materials that inhibited the growth of bacillus causing food poisoning. Also, the decreased inhibition effect is caused by depleting material derived from spent culture, and then it can maintain the growth inhibition effect for bacillus causing food poisoning by using PL bacteria.

TEST 5. Growth inhibition effect for bacillus causing acne

Propionibacterium acne is a normal bacilli existing on skin and causing acne. *Propionibacterium acne* (KCTC 3314) was inoculated in 5 ml of actinomyces broth, and after covering by parapin-oil, *Propionibacterium acne* was anaerobically incubated for 2 days (BR Vowels, S Yang, JJ Leyden. 1995. Induction of proinflammatory cytokines by a soluble factor of *Profionibacterium acnes*: Implications for chronic inflammatory acne. Infection and Immunity 63: 3158-3165). 45 ml of PL spent culture breed on MRS liquid media was mixed with 5 ml of *Profionibacterium acnes* culture solution. The mixture 50 ul was inoculated in 5 ml of fresh media, actinomyces broth and cultured anaerobically for 2 days without shaking. For measurement of survival *Profionibacterium acnes,* culture solution was

diluted (When OD_{600}=2.4, 9.6 X 10^8 CFU/ml) with diluent for anaerobic bacteria containing 0.05 % L-cysteine) and 100 ul of diluted solution was inoculated on actinomyces agar plate. After culture in anaerobic jar, at 37 ℃, for 6-7 days, the number of colony on the plate was measured as the number of survival *Profionibacterium acnes.*

FIG. 12 is a graph showing the growth inhibition activity for bacillus causing acne by PL bacteria, *Lactobacillus coprophilus* PL 9001 did not inhibit nearly the growth of *Profionibacterium acnes, Enterococcus durans* PL 9002 and *Streptococcus faecalis* PL 9003 inhibited completely the growth of *Profionibacterium acnes*, and *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005 and *Lactobacillus fermentum* PL 9006 inhibited the growth of *Profionibacterium acnes.*

TEST 6. Growth inhibition test for anaerobic bacillus.

Clostrodium perfringens was inoculated on BHI liquid broth containing Hemin (0.01 g/L) and L-cysteine (0.5 g/L) and anaerobically cultured at 37 ℃, for 24 hours (Balows A Hausler WJ Herrmann KL Isenberg HD Shadomy HJ. Chapter 50. Clostridium. p505-521. Manual of Clinical Microbiology, 5th ed. ASM Washington D.C. U.S.A.). Spent culture of PL bacteria was mixed with the same volume of 2X concentrated BHI broth and 1 % final concentration of *Clostrodium perfringens* was inoculated on the mixture. After covering mixture with a parapin-oil, it was anaerobically cultured at 37 ℃. After 24 hour, the culture solution was diluted and inoculated on blood agar plate. The plated was incubated for 37 days and

48 days, in anaerobic incubator. The survival *Clostrodium perfringens* was measured and present by log.

[Table 16]

	Number of survival bacteria (log cfu)	
	0 hrs	24 hrs
Clostrodium perfringens	6.60	8.30
Lactobacillus coprophilus PL 9001	6.60	7.60
Enterococcus durans PL 9002	6.78	7.66
Streptococcus faecalis PL 9003	6.60	7.48
Lactobacillus coprophilus PL 9004	6.90	8.00
Lactobacillus fermentum PL 9005	6.60	7.78
Lactobacillus fermentum PL 9006	6.60	7.41

It was confirmed that all of PL bacteria produced materials that inhibited growth of anaerobic bacillus.

TEST 7. Improving effect of immunity

50 ul of coating buffer containing purified rabbit anti-TNF or rabbit anti-IL-6 was coated within a microplate well and the microplate was stayed at –4 ℃ for 12 hr. The microplate well was washed three times with PBST (tween80+phosphate buffer) solution and reacted with 3 % of BSA-PBST, for 30 min. The microplate well was washed four times with PBST and reacted with 50 ul of TNF-standard/IL-6 standard (recombinant mouse TNF-alpha/IL-6, SEROTEC.UK) or supernatant of macrophage cultured in media containing PL bacteria, 37 ℃, for 1 hr. The microplate well was washed four times with PBST, one time with distilled water, and was reacted with 50 ul of mixture prepared by adding 3 % BSA-PBST to biotinylated rat anti-mouse TNF-α or biotinylated rat anti-mouse TNF-alpha/IL-6(1ug/ml), at room temperature for 1 hr. The microplate well was washed six times with

PBST, one time with distilled water, and reacted with streptavidin peroxidase, for one hour. The microplate well was washed eight times with PBST, two times with distilled water, and reacted with TMB substrate (25 ml citric-phosphate buffer+400 ul TMB stock+100 ul of 1 % H_2O_2) at room temperature till showing color. The reaction was stopped to add 6 N of H_2SO_4 and for measurement of immune reactivity, adsorption at OD_{450} was observed.

Therefore, PL bacteria of the present invention can be applied to promote immunity and more particularly, can be used for health food and as a treatment drug that promotes the health for aged persons and children.

TEST 8. Test for acid-resistance and bile-resistance

MRS medium contained cysteine was optimized pH (7, 4.5 or 4.0) with 4 N HCl and 0.1 N NaOH and sterilized. To observe an effect of bile, oxgall powder was added to the medium in 0 %, 0.25 %, 0.50 % conc and sterilized. Each PL bacteria was inoculated medium with total 1 % conc and optical density was determined in 620 nm at 24-hours intervals. Table 17 shows the acid-resistance and bile-resistance. It was shown that PL 9003 was weak in acid, but PL 9001, PL 9002, PL 9004, PL 9005 and PL 9006 had the strong acid-resistant activity and all of PL bacteria had the strong bile-resistant activity. Therefore, all PL bacteria are safe in the stomach and intestine (Conway PL Gorback SL goldin BR. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci. 70: 1-12. : Ibrahim SA Bezkorovainy A. 1993. Survival of bifidobacteria in the

presence of bile salt. J. Sci. Food Agric. 62: 351-354)

[Table 17]

	Time	PL 9001	PL 9002	PL 9003	PL 9004	PL 9005	PL 9006
pH 7	24 hr	1.40	1.20	1.00	1.40	1.50	1.50
	48 hr	1.50	1.50	1.20	1.50	1.50	1.50
pH 5.0	24 hr	1.00	0.70	0.31	0.90	1.50	1.50
	48 hr	1.50	0.80	0.45	1.10	1.50	1.50
pH 4.5	24 hr	0.18	0.21	0.06	0.18	1.50	1.50
	48 hr	0.70	0.28	0.09	0.15	1.50	1.50
Oxgall 0.25 %	24 hr	1.40	1.20	1.00	1.20	1.10	1.20
	48 hr	1.50	1.50	1.40	1.50	1.40	1.20
Oxgall 0.50 %	24 hr	1.10	1.20	1.10	0.85	0.75	0.70
	48 hr	1.40	1.50	1.40	1.00	0.76	0.70

TEST 9. Antibiotic-resistant

An antibiotic-resistant for PL bacteria prepared by the EXAMPLE was observed. PL bacteria was inoculated on MRS solid medium and a filter (diameter 6 mm) containing the antibiotic of Table 18 was put on the medium. After incubation for 24 – 48 h, the diameter of the growth inhibited-zone formed by antibiotics was determined. The decreased diameter of the growth inhibited-zone means that PL bacteria have a resistant to the antibiotics. The result is presented in Table 18.

[Table 18]

Antibiotics	PL9001	PL9002	PL9003	PL9004	PL9005	PL9006
Penicillin (10 IU/E/UI)	20 mm	20 mm	24 mm	23 mm	30 mm	30 mm
Ampicillin (10 ug)	30 mm	24 mm	26 mm	30 mm	30 mm	32 mm
Cephalothin (30 ug)	30 mm	20 mm	20 mm	24 mm	30 mm	30 mm
Gentamycin (10 ug)	13 mm	-	-	12 mm	15 mm	16 mm
Vancomycin (30 ug)	-	20 mm	16 mm	-	-	-
Erythromycin (15 ug)	21 mm	-	-	22 mm	26 mm	29 mm
Tetracycline (30 ug)	26 mm	30 mm	10 mm	24 mm	27 mm	30 mm

As mentioned above, PL bacteria have high antibiotics resistant.

Appl WO 02/45726 file reference OPP010617KR	International application No. PCT/KR01/01286 PCT/KR01/01286

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 4, line 15-16.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

Korea Culture Center of Microorganisms

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongje-1-dong, Sedaemun-gu

SEOUL 120-091 Republic of Korea

Date of deposit	Accession Number
December 2.2000	KCCM - 10246

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus plantarum* PL 9-2	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10246

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2, 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31. 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31. 2001

Form BP/4

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

To be completed in duplicate

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10246

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Enterococcus PL 9002

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus CCARM 9-2

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned 111 ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: Jun. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010617KR	PCT/KR01/01286

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 4, line 16.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

Korea Culture Center of Microorganisms

Address of depositary institution *(including postal code and country)*

361-221, Turim B/D Hongje-1-dong, Seodaemun-gu
SEOUL 120-091 Republic of Korea

Date of deposit	Accession Number
December 2. 2000	KCCM - 10247

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
Issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus paraparacasei* 1 PL 9-3	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10247

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

The microorganism identified under I above was received by this international Depositary Authority on Dec. 2, 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31, 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms

Address : 361-221, Yurim B/D
Hongje-1-dong,
Seodaemun-gu
SEOUL 120-091
Republic of Korea

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) :

Date: Jan. 31, 200

Form BP/4

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM
Accession number given by the INTERNATIONAL DEOSITARY AUTHORITY: KCCM 10247
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]
☐[2] Scientific description:
☐[2] Last preceding scientific description (if any):
☐[2] Proposed taxonomic designation: Enterococcus PL 9003
☐[2] Last preceding proposed taxonomic designation (if any): Lactobacillus CCARM 9-3

Mark with a cross if additional information is given on an attached sheet.

Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned [1] ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonbee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP 010617KR	PCT/KR01/01286

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 4, line 15.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL 120-091 Republic of Korea

Date of deposit	Accession Number
December 21 2000	KCCM - 10245

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus coprophilus* PL 9-1	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10245

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2, 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31. 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31. 200

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.

INDUSTRIAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms
Address : 361-221, Yurim B/D
Hongje-1-dong,
Seodaemun-gu,
SEOUL 120-091
Republic of Korea

Signature(s) of person(s) having the power to *represent* the International Depositary Authority or authorized official(s) :

Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TRRATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

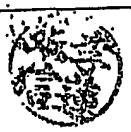
TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM
Accession number given by the INTERNATIONAL DEOSITARY AUTHORITY: KCCM 10245
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]
☐[2] Scientific description:
☐[2] Last preceding scientific description (if any):
☐[2] Proposed taxonomic designation: Lactobacillus PL 9001
☐[2] Last preceding proposed taxonomic designation (if any): Lactobacillus CCARM 9-1

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned 111 ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010617KR	PCT/KR01/01286

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 4, line 17.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution

KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongje-1-dong, Sedaemun-gu

SEOUL 120-091 Republic of Korea

Date of deposit	Accession Number
December 2. 2000	KCCM -10248

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I .IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus coprophilus* PL 9-4	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10248

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2. 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31. 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31. 2001

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.

INDUSTRIAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms
Address : 361-221, Yurim B/D
Hongje-1-dong,
Seodaemun-gu,
SEOUL 120-091
Republic of Korea

Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) :

Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul, 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEPOSITARY AUTHORITY:
KCCM 10248

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Lactobacillus PL 9004

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus CCARM 9-4

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

III. REQUEST FOR ATTESTATION	
The undersigned III ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee Lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010617KR	PCT/KR01/01286

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 4, line 18.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL 120-091
Republic of Korea

Date of deposit	Accession Number
Mar. 2. 2001	KCCM-10250

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeonhee Lee
Department of Biology,
Seoul Woman's University,.
Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus* PL 9-5	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM-10250
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on Mar. 2, 2001. (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of of authorized official(s): Date: Mar. 8. 2001.

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee Ice
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) : Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10250

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Lactobacillus PL 9005

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus PL 9-5

Mark with a cross if additional information is given on an attached sheet.

Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned 111 ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010617KR	PCT/KR01/01286

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 4, line 18.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

Korea Culture Center of Microorganisms

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongjr-1-dong, Seodaemun-gu
SEOUL 120-091 Republic of Korea

Date of deposit	Accession Number
Mar.2.2001.	KCCM - 10251

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeonhee Lee
Department of Biology,
Seoul Woman's University,
Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus* PL 9-6	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM-10251
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on Mar. 2. 2001. (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 *Republic of Korea*	Signature(s) of person(s) having the power to represent the International Depositary Authority of of authoulzed official(s) : Date: Mar. 8. 2001.

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of I nternational depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authouity.

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) : Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

To be completed in duplicate

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10251

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Lactobacillus PL 9006

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus PL 9-6

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

WHAT IS CLAIMED IS:

1. A lactic acid bacteria containing inhibiting activity against the adherence of *Helicobacter pylori* of the gastric mucous membrane.

2. The lactic acid bacteria of claim 1, wherein the lactic acid bacteria are *Lactobacillus* sp. or *Enterococcus* sp.

3. The lactic acid bacteria of claim 1, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus*, *Enterococcus durans, Streptococcus faecalis,* and *Lactobacillus fermentum.*

4. The lactic acid bacteria of claim 1, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247) *Lactobacillus coprophilus* PL 9004(KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

5. The lactic acid bacteria of claim 1, wherein the lactic acid bacteria are raw, dehydrated, or dead.

6. A medicine for preventing or treating infectious bacteria containing lactic acid bacteria.

7. The medicine for preventing or treating infectious bacteria of claim 6, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus*, *Enterococcus durans*, *Streptococcus faecalis, and Lactobacillus fermentum*.

8. The medicine for preventing or treating infectious bacteria of claim 6, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247) *Lactobacillus coprophilus* PL 9004(KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

9. The medicine for preventing or treating infectious bacteria of claim 6, wherein the infectious bacteria are *Helicobacter pylori*, bacillus causing food poisoning, bacillus causing acne, or anaerobic bacteria.

10. The medicine for preventing or treating infectious bacteria of claim 6, wherein the lactic acid bacteria are raw lactic acid bacteria, fragment of lactic acid bacteria cell wall or spent culture of lactic acid bacteria.

11. A cosmetic composition containing lactic acid bacteria or spent culture of lactic acid bacteria.

12. The cosmetic composition of claim 11, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus*, *Enterococcus durans*, *Streptococcus faecalis, and Lactobacillus fermentum*.

13. The cosmetic composition of claim 11, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247) *Lactobacillus*

coprophilus PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250, and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

14. The cosmetic composition of claim 11, wherein the cosmetic composition is for external application against infectious disease.

15. The cosmetic composition of claim 11, wherein the cosmetic composition is for treatment of acne.

16. A food additive containing lactic acid bacteria or spent culture of lactic acid bacteria.

17. The food additive of claim 16, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus*, *Enterococcus durans*, *Streptococcus faecalis, and Lactobacillus fermentum.*

18. The food additive of claim 16, wherein, the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247) *Lactobacillus* coprophilus PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250, and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

19. The food additive according to claim 16, wherein the food additive is applied to yogurt, baby food, dairy goods, cheese, Kimchi, drinks, or food.

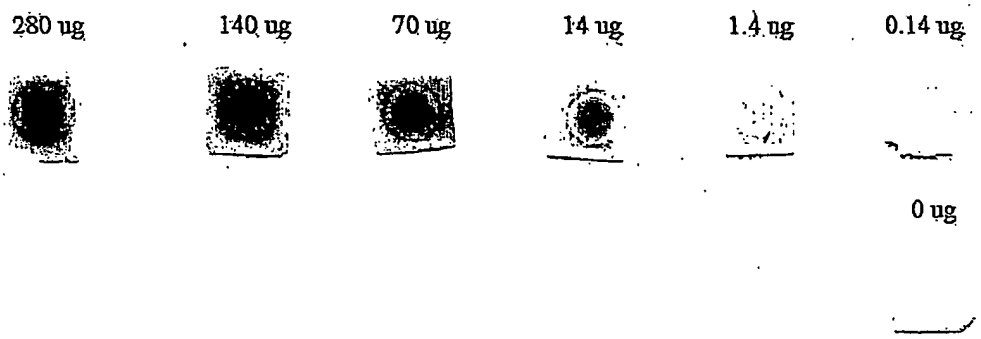
20. A food prepared by fermenting with lactic acid bacteria.

21. The food of claim 20, wherein the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus*,

Enterococcus durans, *Streptococcus faecalis, and Lactobacillus fermentum*.

22. The food of claim 20, wherein the lactic acid bacteria are the lactic acid bacteria are at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247) *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250, and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

FIG.1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70 ug
14 ug
1.4 ug
0.14 ug
0 ug

FIG.2

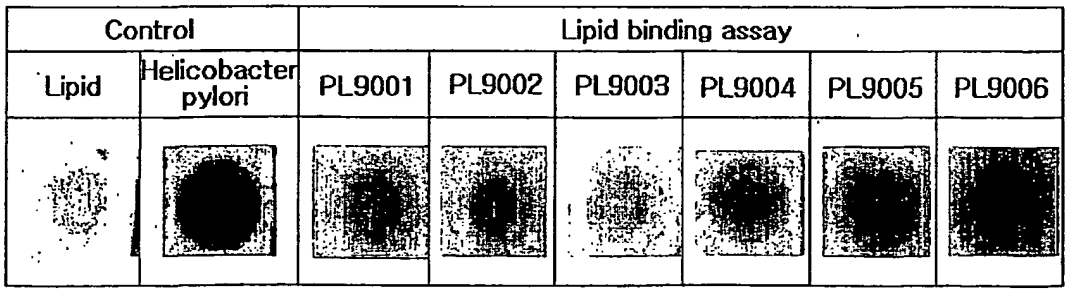
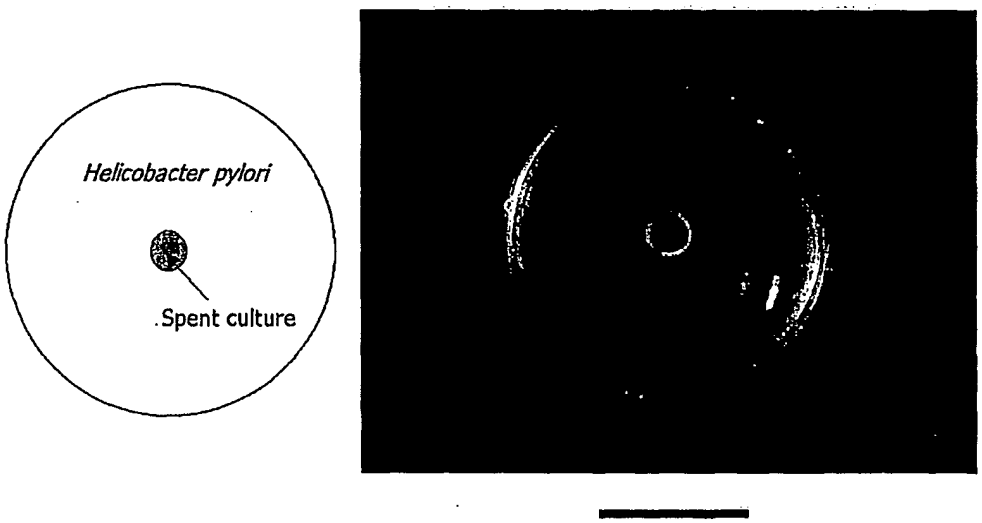

Control
Lipid binding assay
Lipid
Helicobacter pylori
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006

FIG.3



(Diamter of Growth-Inhibited-zone)

FIG.4

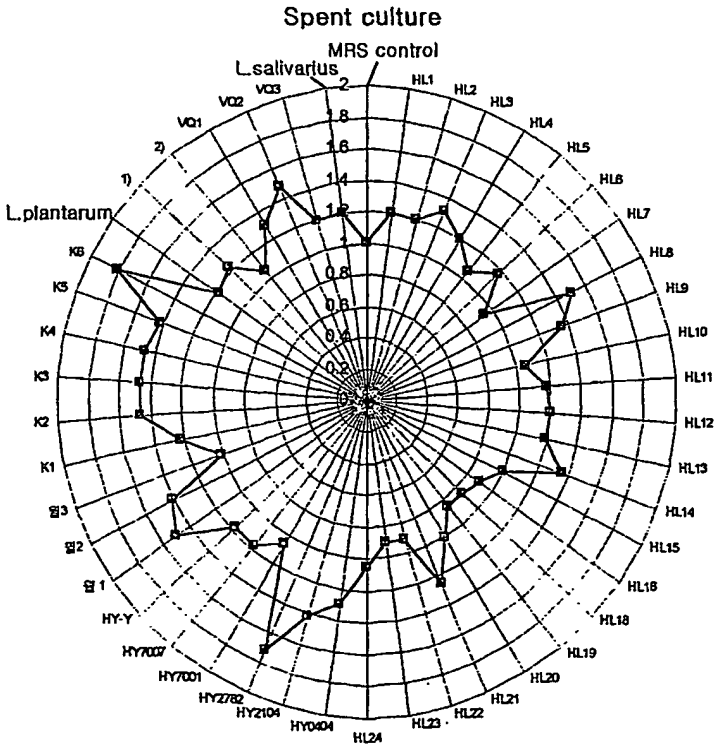


FIG.5

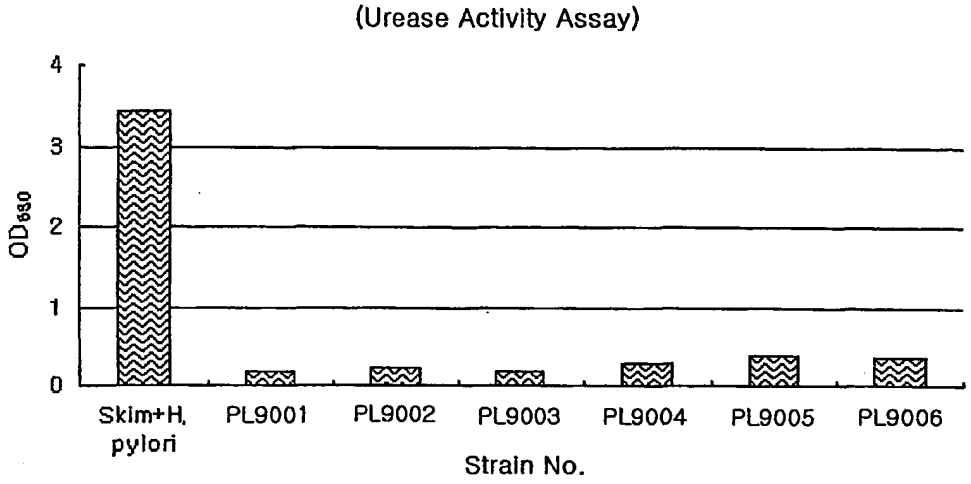
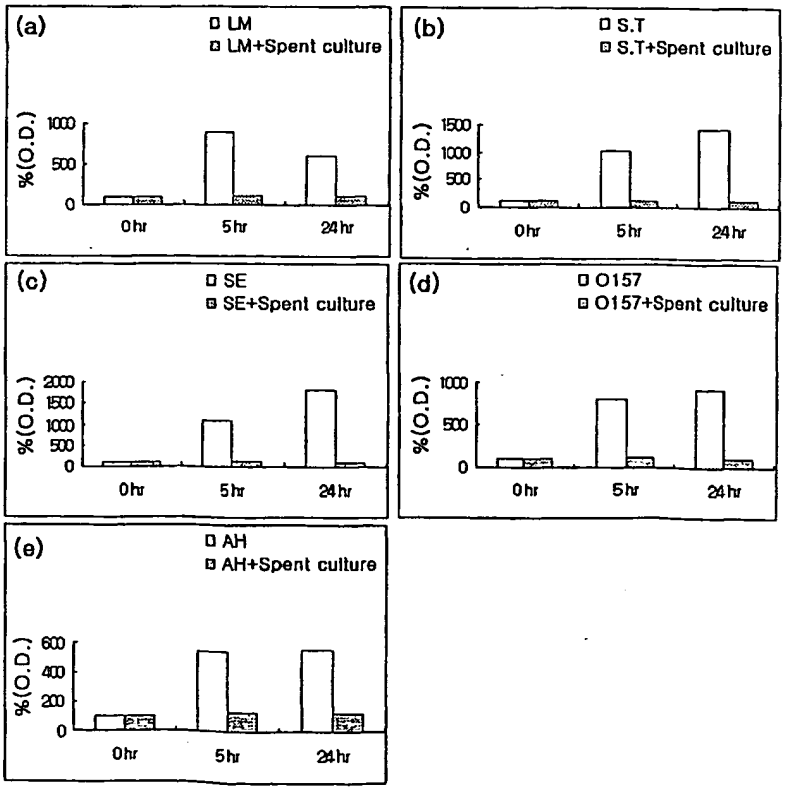

(Urease Activity Assay)
OD650
4
3
2
1
0
Skim+H. pylori
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006
Strain No.

FIG.6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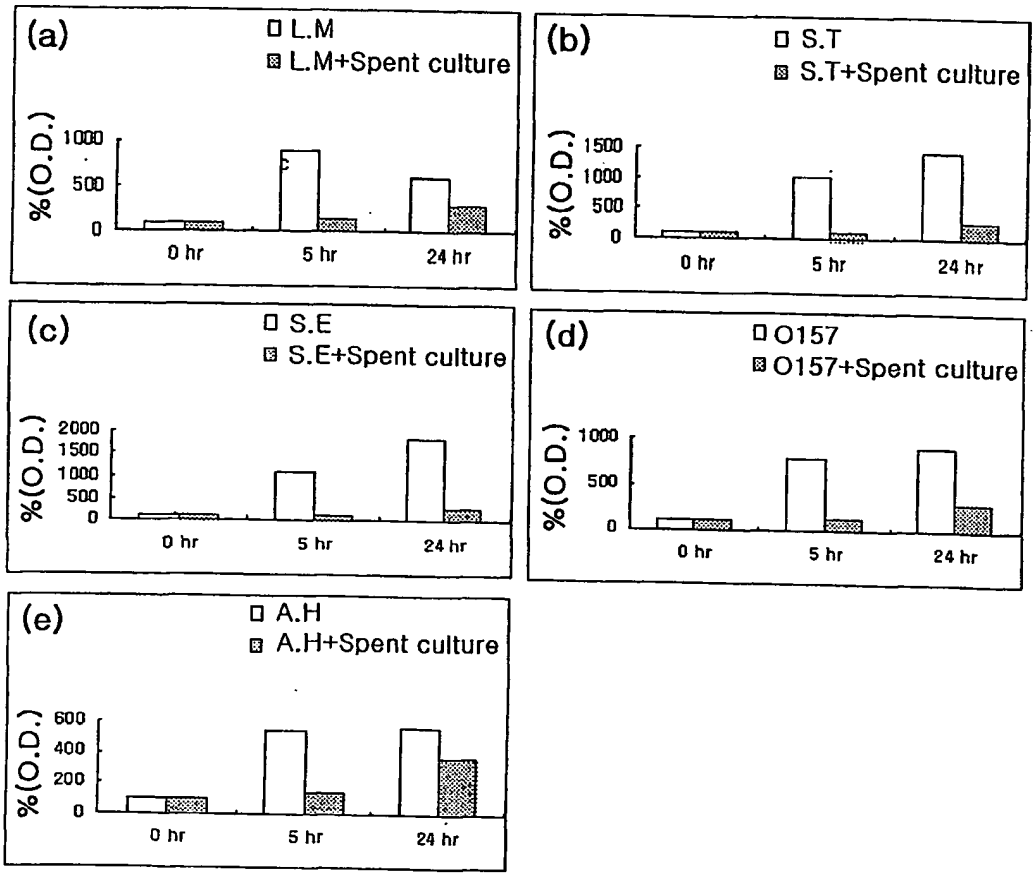
LM
LM+Spent culture
%(O.D.)
1000
500
0
0hr
5hr
24hr
(b)
S.T
S.T+Spent culture
%(O.D.)
1500
1000
500
0
0hr
5hr
24hr
(c)
SE
SE+Spent culture
%(O.D.)
2000
1500
1000
500
0
0hr
5hr
24hr
(d)
O157
O157+Spent culture
%(O.D.)
1000
500
0
0hr
5hr
24hr
(e)
AH
AH+Spent culture
%(O.D.)
600
400
200
0
0hr
5hr
24hr

FIG.7


(a)
L.M
L.M+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(b)
S.T
S.T+Spent culture
%(O.D.)
1500
1000
500
0
0 hr
5 hr
24 hr
(c)
S.E
S.E+Spent culture
%(O.D.)
2000
1500
1000
500
0
0 hr
5 hr
24 hr
(d)
O157
O157+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(e)
A.H
A.H+Spent culture
%(O.D.)
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400
200
0
0 hr
5 hr
24 hr

FIG.8

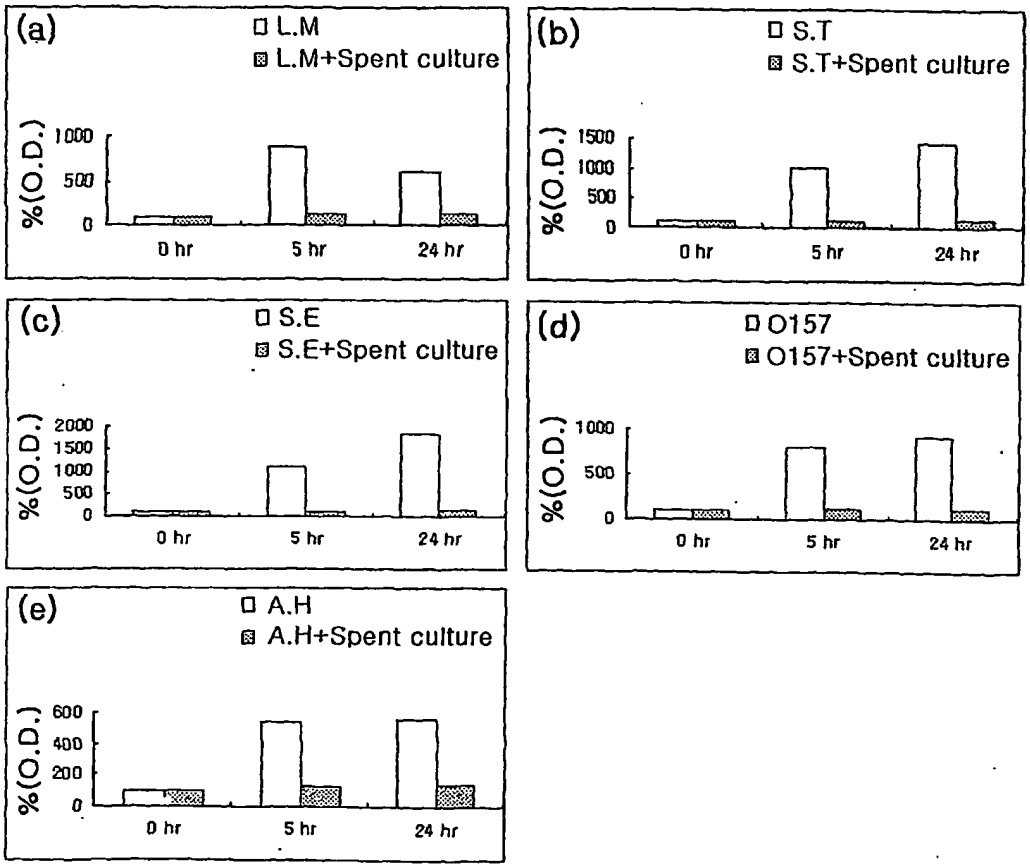
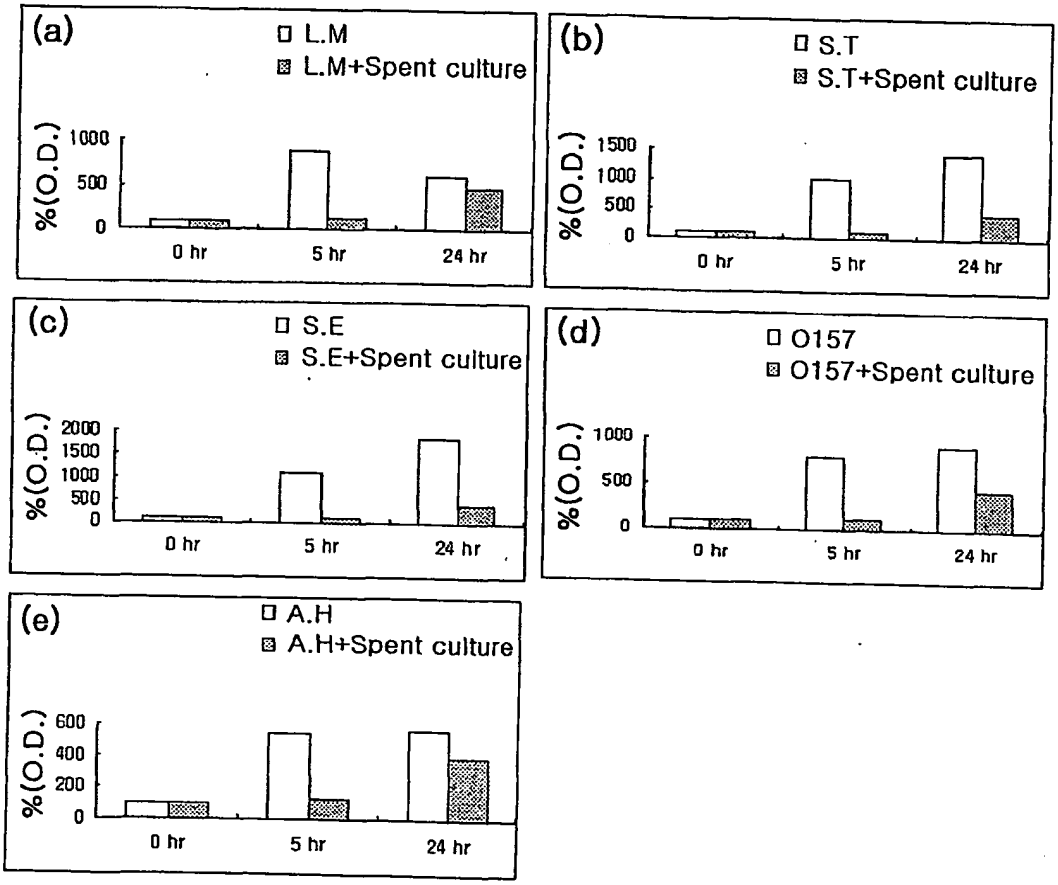

(a)
L.M
L.M+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(b)
S.T
S.T+Spent culture
%(O.D.)
1500
1000
500
0
0 hr
5 hr
24 hr
(c)
S.E
S.E+Spent culture
%(O.D.)
2000
1500
1000
500
0
0 hr
5 hr
24 hr
(d)
O157
O157+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(e)
A.H
A.H+Spent culture
%(O.D.)
600
400
200
0
0 hr
5 hr
24 hr

FIG.9


(a)
L.M
L.M+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(b)
S.T
S.T+Spent culture
%(O.D.)
1500
1000
500
0
0 hr
5 hr
24 hr
(c)
S.E
S.E+Spent culture
%(O.D.)
2000
1500
1000
500
0
0 hr
5 hr
24 hr
(d)
O157
O157+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(e)
A.H
A.H+Spent culture
%(O.D.)
600
400
200
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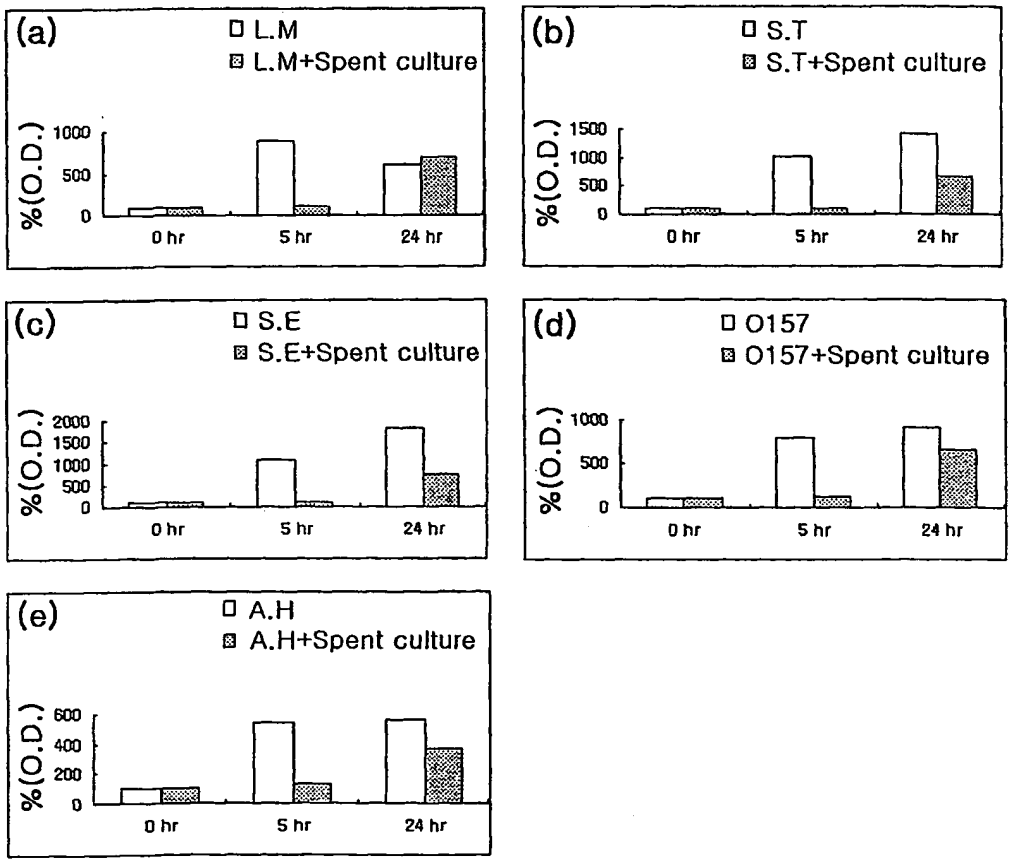
5 hr
24 hr

FIG.10


(a)
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L.M+Spent culture
%(O.D.)
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500
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0 hr
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24 hr
(b)
S.T
S.T+Spent culture
%(O.D.)
1500
1000
500
0
0 hr
5 hr
24 hr
(c)
S.E
S.E+Spent culture
%(O.D.)
2000
1500
1000
500
0
0 hr
5 hr
24 hr
(d)
O157
O157+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(e)
A.H
A.H+Spent culture
%(O.D.)
600
400
200
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0 hr
5 hr
24 hr

FIG.11

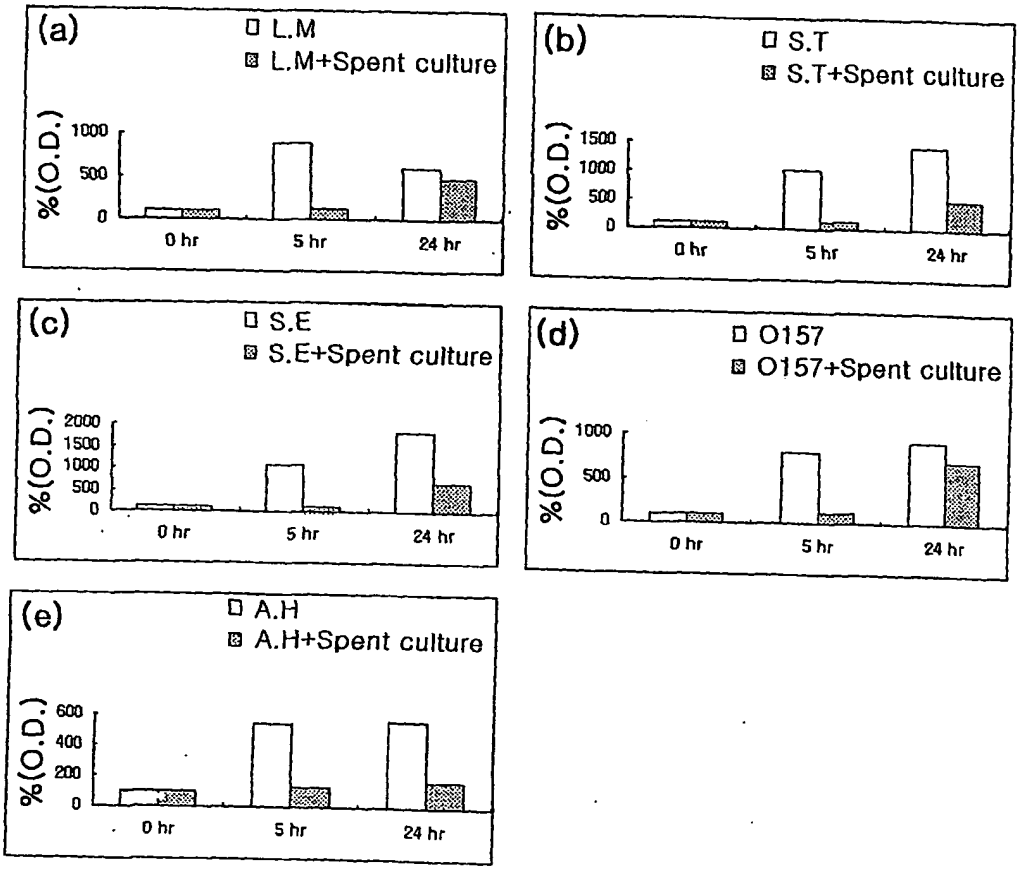

(a)
L.M
L.M+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(b)
S.T
S.T+Spent culture
%(O.D.)
1500
1000
500
0
0 hr
5 hr
24 hr
(c)
S.E
S.E+Spent culture
%(O.D.)
2000
1500
1000
500
0
0 hr
5 hr
24 hr
(d)
O157
O157+Spent culture
%(O.D.)
1000
500
0
0 hr
5 hr
24 hr
(e)
A.H
A.H+Spent culture
%(O.D.)
600
400
200
0
0 hr
5 hr
24 hr

FIG.12

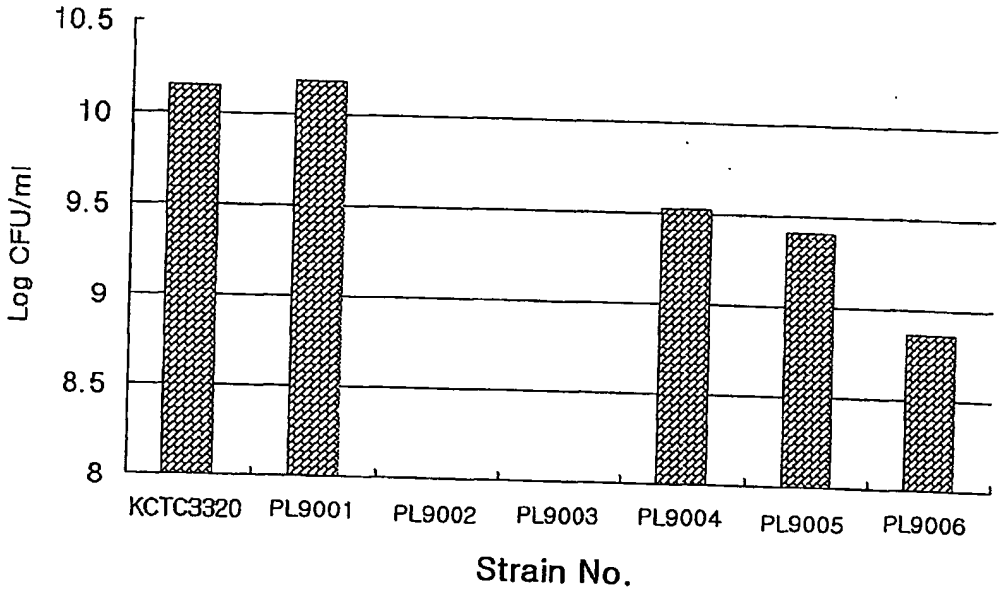
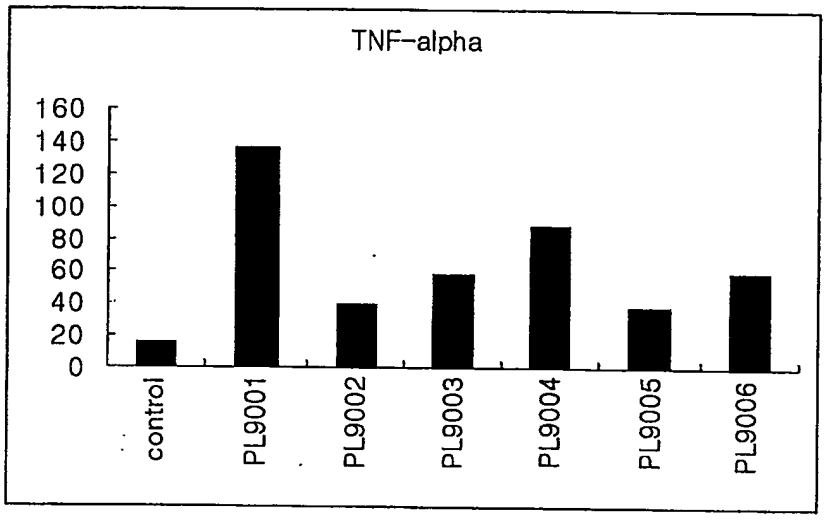

10.5
10
9.5
9
8.5
8
Log CFU/ml
KCTC3320
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006
Strain No.

FIG.13


TNF-alpha
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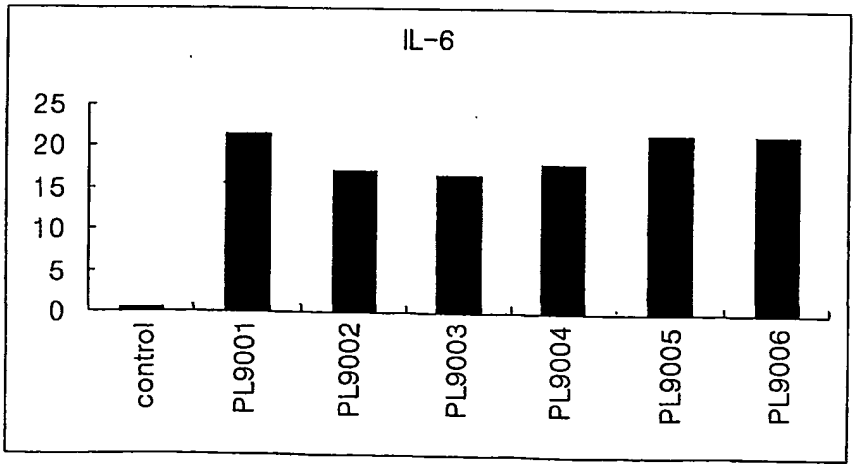
80
60
40
20
0
control
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006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15
10
5
0
control
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006

SEQUENCE LISTING

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<130> opp010617kr

<150> KR10-2000-74602
<151> 2000-12-08

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<151> 2001-1-17

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<151> 2002-2-20

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<151> 2001-7-5

<160> 6

<170> KopatentIn 1.71

<210> 1

<211> 527

<212> DNA

<213> 16S rRNA sequence of *Lactobacillus coprophilus* PL 9001

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gaaacctacc tcttagcagg ggataacatt tggaaacaga tgctaatacc gtataacaat 180

agcaaccgca tggttgctac ttaaaagatg gttctgctat cactaagaga tggtcccgcg 240

gtgcattagt tagttggtga ggtaatggct caccaagacg atgatgcata gccgagttga 300

gagactgatc ggccacaatg ggactgagac acggcccata ctcctacggg aggcagcagt 360

agggaatctt ccacaatggg cgaaagcctg atggagcaac gccgcgtgtg tgatgaaggg 420

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gtgacggtat cttaccagaa aggaacggct aaatacgtgc cagcaag 527

<210> 2

<211> 481

<212> DNA

<213> 16S rRNA sequence of *Enterococcus durans* PL 9002

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ctccaccgga aaaagaagag tggcgaacgg gtgagtaaca cgtgggtaac ctgcccatca 120

gaaggggata acacttggaa acaggtgcta ataccgtata acaatcgaaa ccgcatggtt 180

ttgatttgaa aggcgctttc gggtgtcgct gatggatgga cccgcggtgc attagctagt 240

tggtgaggta acggctcacc aaggccacta tgcatagccg acctgagagg gtgatcggcc 300

acattgggac tgagacacgg cccaaactcc tacgggaggc agcagtaggg aatcttcggc 360

aatggacgaa agtctgaccg agcaacgccg cgtgagtgaa gaaggttttc cgggaatcgt 420

taaactctgt ttgttaagaa gaaagaacca aaggggattg aaggagttat cttgtttcat 480

t 481

<210> 3

<211> 825

<212> DNA

<213> 16S rRNA sequence of *Streptococcus faecalis* PL 9003

<400> 3

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tggaaagagg agtggcggac gggtgagtaa cacgtgggta acctacccat cagaggggga 120

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aaaggcgctt tcgggtgtcg ctgatggatg gacccgcggt gcattagcta gttggtgagg 240

taacggctca ccaaggccac gatgcatagc cgacctgaga gggtgatcgg ccacactggg 300

actgagacac ggcccagact cctacgggag gcagcagtag ggaatcttcg gcaatggacg 360

aaagtctgac cgagcaacgc cgcgttgagt gaaagaaggt ttttcgggat cgttaaaaac 420

tctgttgttt agaggaatct tcggatggac gaaagctgac cgagcaacgc cgcgtgagtg 480

aagaaggttt tcggatcgta aaactctgtt gttagagaag aacaaggacg ttagtaactg 540

aacgtcccct gacggtatct aaccagaaag ccacggctaa ctacgtgcca gcagccgcgg 600

taatacgtag gtggcaagcg ttgtccggat ttattgggcg taaagcgagc gcaggcggtt 660

tcttaagtct gatgtgaaag cccccggctc aaccggggag ggtcattgga aactgggaga 720

cttgagtgca gaagaggaga gtggaattcc atgtgtagcg gtgaaatgcg tagatatatg 780

gaggaacacc agtggcgaag gcggctctct ggtctgtaac tgacg 825

<210> 4

<211> 475

<212> DNA

<213> 16S rRNA sequence of *Lactobacillus coprophilus* PL 9004

<400> 4

acgctggcgg cgtgcctaat acatgcaagt cgaacgcttt gtggttcaac tgatttgaag 60

agcttgctca gatatgacga tggacattgc aaagagtggc gaacgggtga gtaacacgtg 120

ggaaacctac ctcttagcag gggataacat ttggaaacag atgctaatac cgtataacaa 180

tagcaaccgc atggttgcta cttaaaagat ggttctgcta tcactaagag atggtcccgc 240

ggtgcattag ttagttggtg aggtaatggc tcaccaagac gatgatgcat agccgagttg 300

agagactgat cggccacaat gggactgaga cacggcccat actcctacgg gaggcagcag 360

tagggaatct tccacaatgg gcgaaagcct gatggagcaa cgccgcgtgt gtgatgaagg 420

gtttcggctc gtaaaacact gttgtaagag aagaatgaca ttgagagtaa ctgtt 475

<210> 5

<211> 339

<212> DNA

<213> 16S rRNA sequence of *Lactobacillus fermentum* PL 9005

<400> 5

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tgattgattt tggtcgccaa cgagtggcgg acgggtgagt aacacgtagg taacctgccc 120

agaagcgggg gacaacattt ggaaacagat gctaataccg cataacagcg ttgttcgcat 180

gaacaacgct taaaagatgg cttctcgcta tcacttctgg atggacctgc ggtgcattag 240

cttgttggtg gggtaacggc ctaccaaggc gatgatgcat agccgagttg agagactgat 300

cggccacaat gggactgaga cacggcccat actcctacg 339

<210> 6

<211> 447

<212> DNA

<213> 16S rRNA sequence of *Lactobacillus fermentum* PL 9006

<400> 6

acgccggcgg tgtgcctaat acatgcaagt cgaacgcgtt ggcccaattg attgatggtg 60

cttgcacctg attgattttg gtcgccaacg agtggcggac gggtgagtaa cacgtaggta 120

acctgcccag aagcggggga caacatttgg aaacagatgc taataccgca taacagcgtt 180

gttcgcatga acaacgctta aaagatggct tctcgctatc acttctggat ggacctgcgg 240

tgcattagct tgttggtggg gtaanggcct accaaggcga tgatgcatag ccgagttgag 300

agactgatcg gccacantgg gactgagaca cggcccatac tcctacggga ggcagcagta 360

gggaatcttc cacaatgggc gcaagcctga tggagcaaca ccgcgtgagt gaagaagggt 420

ttcggctcgt aaagctctgt tgttaaa 447

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/01286

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

There are found three inventions:

1. Claims 1 - 10 concern lactic acid bacteria containing inhibiting activity against H. pylori and the use of these bacteria as a medicine.

2. Claims 11 - 15 concern cosmetic compositions containing lactic acid bacteria.

3. Claims 16 - 22 relate to food and food additives containing lactic acid bacteria.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/01286

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: A61K 35/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: A61K 35/74

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/55131 A1 (GRAHN HAKANSSON, E. et al.) 10 December 1998 (10.12.98) *page 4, lines 19-36; page 6, lines 17-30; page 7, lines 3-25; page 9, lines 8-12.*	1-3,5-7,9,10,16,17,19-21
X	EP 1000625 A1 (LABORATOIRE DU LACTEOL DU DOCTEUR BOUCARD, INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) 17 May 2000 (17.05.00) *abstract; page 1, lines 25-27; claims 1,3,4,6.*	1-3,5-7,9,10
X	EP 1038951 A1 (SOCIETE DES PRODUITS NESTLE S.A.) 27 September 2000 (27.09.00) *page 1, lines 6-10, 51-54.*	1-3,5-7,9,10,16,17,19-21
X	JP 09 241173 A (WAKAMOTO PHARM CO LTD) 16 September 1997 (16.09.97) (abstract) WPI [online]. London, U.K.: Derwent Publications, Ltd. [retrieved on 08-10-2001]. DW 199750, AN: 1997-539731 [50] *abstract.*	1-3,5-7,9,10,16,17,19-21

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 1998 (08.10.1998)	6 December 2001 (06.12.2001)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer MOSSER Telephone No. 1/53424/437

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/01286

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 63179829 A (ADVANCE KK) 23 July 1988 (23.07.88) (abstract) WPI [online]. London, U.K.: Derwent Publications, Ltd. [retrieved on 08-10-2001]. DW198835, AN: 1988-246702 [25] *abstract.*	11,12,14,15

INTERNATIONAL SEARCH REPORT
Information on patent family members

Intc nal application No.
PCT/KR 01/01286

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
EP	A1	1000625	17-05-2000	FR	A1	2785190	05-05-2000
				FR	B1	2785190	26-01-2001
EP	A1	1038951	27-09-2000	BR	A	00001392	02-05-2001
				JP	A2	00279166	10-10-2000
JP	A2	9241173	16-09-1997			none	
JP	A2	63179829	23-07-1988	JP	B4	8018995	28-02-1996
WO	A1	9855131	10-12-1998	AU	A1	80460/98	21-12-1998
				AU	B2	726217	02-11-2000
				CN	T	1272791	08-11-2000
				EP	A1	1005353	07-06-2000
				NO	A0	995794	26-11-1999
				NO	A	995794	31-01-2000
				SE	A0	9702083	02-06-1997
				SE	A	9702083	03-12-1998
				SE	C2	511524	11-10-1999